Titanium dioxide nanoparticles reduce human sperm DNA stability

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Introduction

The environmental release of nanoparticles (< 100 nm) due to industrial, commercial and domestic waste, is on the rise given their increased global use and has been implicated in poor human sperm functionality decreasing the rate of fertilization. Nanoparticles, in fact, can penetrate the blood-testis barrier, acting at various biological levels, inducing inflammation and so could contribute to alter reproductive functions [1]. Nanosized titanium dioxide (n-TiO_2) (Fig. 1) is one of the most widely used nanomaterials (Fig. 2). Considering the large scale of production and use of this nanomaterial, there has recently been much interest as regards the safety of n-TiO_2, the implications of its emission into the environment and the possible effects on human reproductive health.

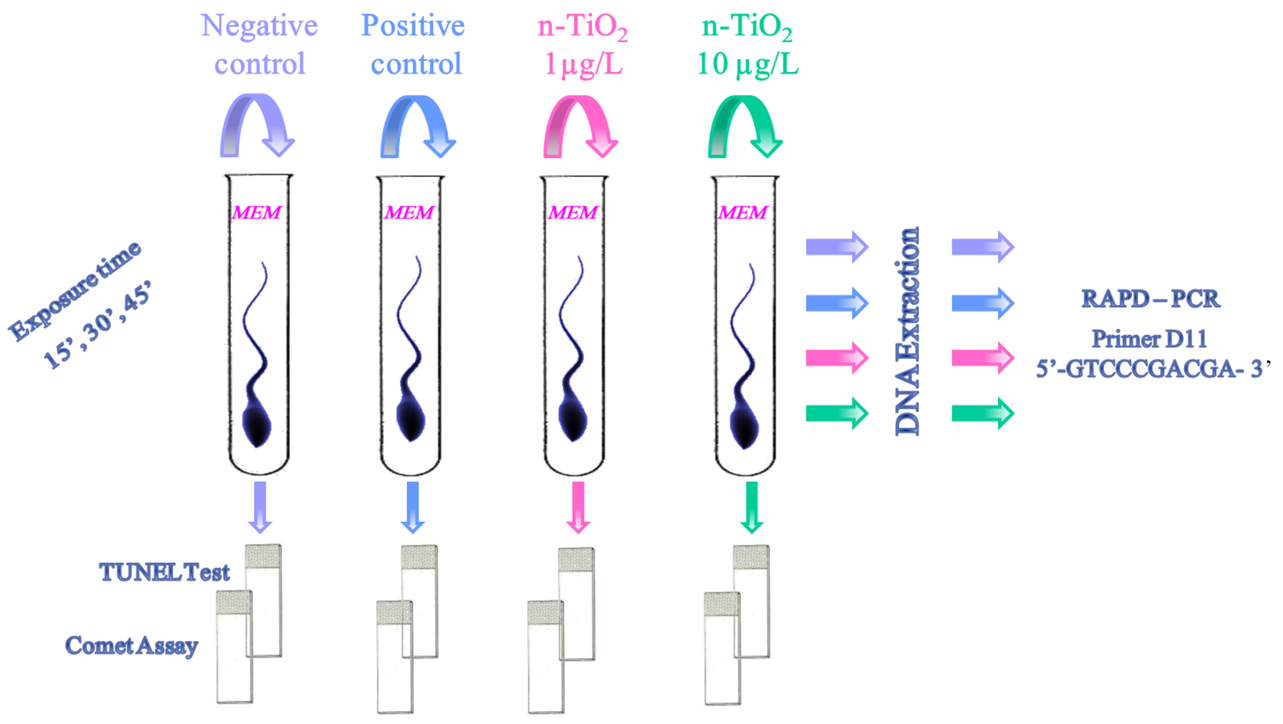
Fig. 1. *Nanosized titanium dioxide powder.*

Fig. 2. *Commercial products containing nanosized titanium dioxide.*

Study Question

To date there are no data in the literature on the effects on human sperm DNA induced *in vitro* by n-TiO_2. The aim of this study was to investigate the DNA damage and the variation in genomic stability in human sperm exposed *in vitro* to two concentrations of n-TiO_2 (1 µg/L and 10 µg/L) for three different times (15, 30 and 45 minutes). The genotoxicity was evaluated by means of the Comet Assay, the TUNEL test and the RAPD-PCR technique with relative quantitative analyses (Genomic Template Stability, GTS %).

Study design



Material & Methods

Sample collection and exposure procedure.
From April to November 2014, they were selected 76 human semen samples with good parameters in sperm quality according to WHO guidelines 2010. The samples, after selection with discontinuous Percoll gradient (45% and 90%), were exposed *in vitro* to 1 µg/L and 10 µg/L of n-TiO_2 for 15, 30 and 45 minutes at 37° C. One aliquot was treated with 0,4 µl/L benzene (positive control) and an untreated aliquot was used as negative control. The concentrations of n-TiO_2 were selected according to the concentration values present in the environment [2]. Stock solutions of dispersed n-TiO_2 P25 were prepared by sonication without using solvents and were dosed according to Rocco et al. (2015) [3].

Genotoxicity tests.
The Comet Assay evaluated DNA integrity at single and double breaks strands (Fig. 3a), while TUNEL test evaluated the number of apoptotic cells expressed like a percentage of DNA Fragmentation Index (% DFI) (Fig. 4a). The RAPD-PCR technique was used to calculate the GTS % considering the total number of bands present in the negative control (*n*) (set as 100%) and the number of polymorphisms present in the treated samples (*a*).

$$\%GTS = \left(1 - \frac{a}{n}\right) \times 100$$

Statistical Analysis.
Differences in the percentage of DNA damage among the experimental groups were analyzed using the unpaired Student's t-test. In all analysis, the effect was considered significant if p-value ≤ 0.05. The statistical analysis for GTS% were carried out using the software package SPSS 9.05 for Windows.

Results

The exposure to 1 µg/L and 10 µg/L n-TiO_2 for 15, 30 and 45 minutes did not induce a statistically significant increase in motility, viability and anomalies of the head, tail and intermediate tract. The Comet Assay showed a statistically significant loss (p-value ≤ 0.05) of sperm DNA integrity already after 15' of exposure for both concentrations tested (Fig. 3b). The results of the TUNEL test showed an increase in sperm DNA fragmentation (Fig. 4b) but each value of DFI% observed was under 27% that is considered pathologic and may cause failed conception in many couples [4]. The RAPD-PCR analysis showed a variation of the polymorphic profiles of the sperm DNA exposed to n-TiO_2 respect to the DNA of the not-treated sperms with a consequent reduction of sperm DNA stability (Fig. 5). The DNA damage was greatest at the highest concentration of n-TiO_2.

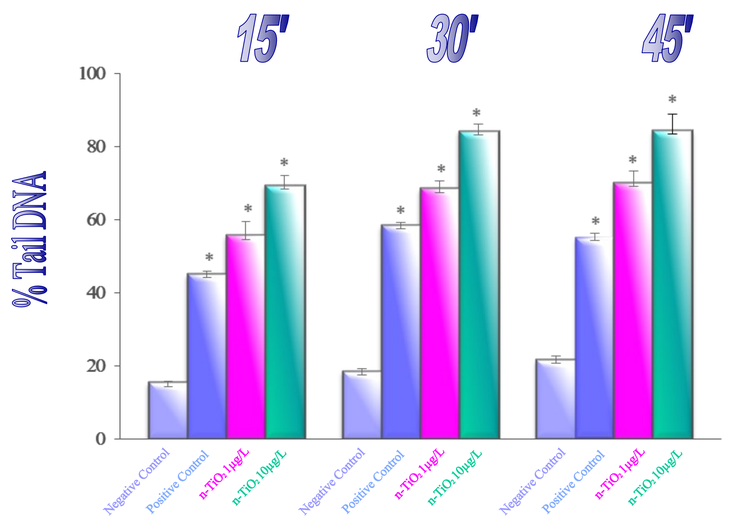


Fig. 3b. *Percentage of DNA in the tail of the comets(ordinate) in human sperm after different times of exposure to 1 µg/L and 10 µg/L of n-TiO_2 (abscissa). The values are expressed as avarage ± SD. *p ≤ 0.05.*

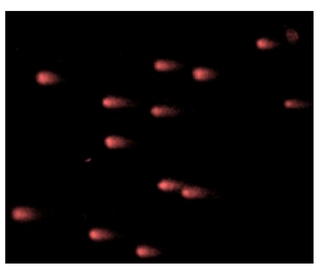


Fig. 3a. *Tail DNA in human sperm cells analyzed using the Komet 5.5 Software.*

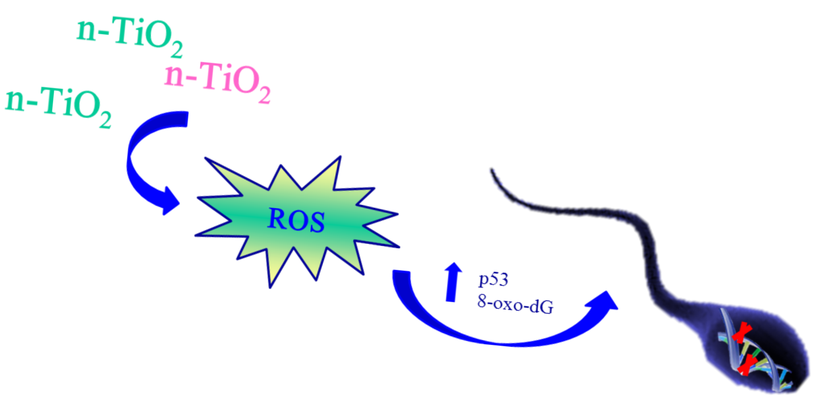


Fig. 6. *n-TiO_2 genotoxic activity in sperm.*

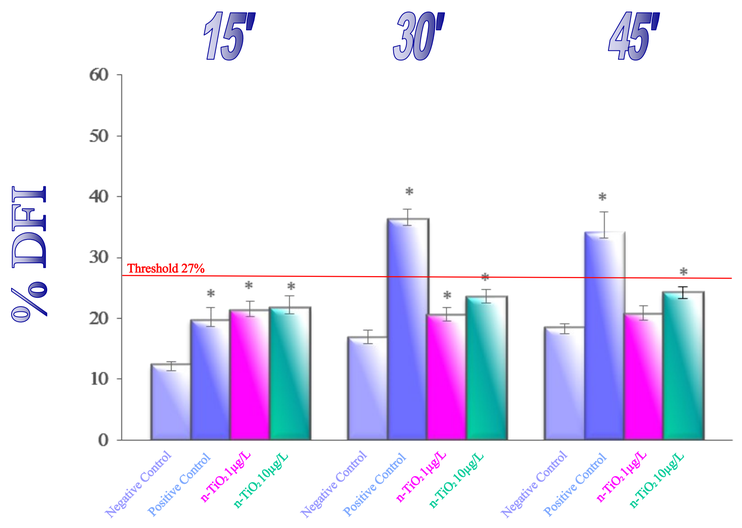


Fig. 4b. *Percentage of DNA index fragmentation (ordinate) in human sperm after different times of exposure to 1 µg/L and 10 µg/L of n-TiO_2 (abscissa). The DFI% are expressed as avarage ± SD. *p ≤ 0.05.*

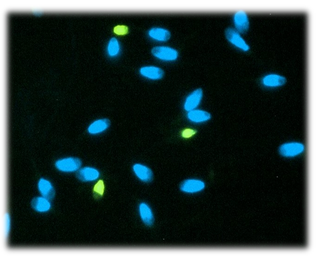


Fig. 4a. *Apoptosis in human sperm cells analyzed using the TUNEL test. Apoptotic cells appear green compared with blue non-apoptotic cells.*

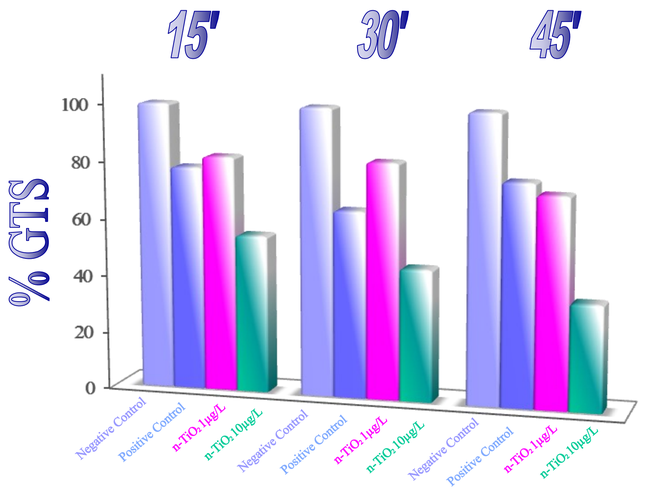


Fig. 5. *Changes in percentage of Genome Template Stability (ordinate) in human sperm DNA exposed to 1 µg/L and 10 µg/L of n-TiO_2 for different exposure times (abscissa) as evidenced by RAPD-PCR technique.*

Discussion & Conclusions

This research provides the first data on the evaluation of the potential genotoxicity of n-TiO_2 on human seminal liquid. Thanks to the qualitative analysis of the RAPD profiles, we hypothesized that the damage to sperm DNA induced by n-TiO_2 occurs through the production of reactive oxygen species (ROS) linked to point mutations (deletion or insertion of a base) and DNA rearrangements [5] (Fig.6). TUNEL test evidenced values of DFI% under the cut-off value related to male infertility (27%). On the other side, Comet Assay demonstrated a statistically significant increase of DNA strand breaks and RAPD-PCR technique evidenced a decrease in sperm genomic stability after the exposure to n-TiO_2. So we believe that the concentrations of n-TiO_2 tested are not able to trigger the apoptotic process, but nevertheless cause an injury to DNA highlighted by the Comet Assay and the RAPD-PCR technique. Then the n-TiO_2 caused *in vitro* an earlier stage of human sperm cell suffering inducing DNA instability before it can generate the apoptotic process. These data provide a starting point for investigations on the possible effects that other nanomaterials could have on human sperm DNA and their incidence on infertility in the couple and spontaneous abortions also after *in vitro* fertilization techniques.

Reference

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